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Future directions in phosphodiesterase drug discovery

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ABSTRACT

Research on phosphodiesterases both in academic labs and in the pharmaceutical industry has remained steady over the past 35 years. Although there have been some clinical successes, they have been clustered around just a couple of PDE isoforms, and disproportionate to the huge investment put forth against what seem like very druggable targets. This review attempts to uncover the reasons for the lack of productivity in PDE drug discovery, and summarizes the current hot areas of research. In addition, new insights gathered about structure–function relationships are highlighted, in particular those relating to enzyme regulation. Lastly, novel strategies for targeting the activation or inactivation of selected PDEs are proposed that may allow for a more targeted approach for PDE modulation.

Phosphodiesterases (PDEs) hydrolyze two of the most important signaling molecules in cells, cAMP and cGMP. These important biomolecules are involved in many signaling processes, particularly those emanating from the extracellular activation of membrane proteins to the internal machinery of the cell. As such, mammals have evolved intricate systems for controlling localized intracellular concentrations of these molecules. These systems include pathways to increase levels through synthesis (via adenylate cyclase and guanylate cyclase) or to facilitate their disposal either through hydrolysis by PDEs, or by active transport out of the cell.[1] The inhibition of PDEs has remained an active area of drug research for over thirty years.[2] During that time, advances in biology has identified additional subtypes, and has enabled a greater understanding of the tissue distribution and function of this class of enzymes.[3,4] There are a total of 21 PDE isoforms grouped into 11 families. The majority of the PDE families (PDE1, 2, 3, 10 and 11) are dual-substrate and can hydrolize both cAMP and cGMP although not at similar rates. PDE4, 7 and 8 are cAMP specific while PDE5, 6, and 9 only break down cGMP. They differ in their substrate specificity and affinity, regulatory mechanisms and tissue distribution.[5] To address the ever increasing level of complexity in the field, scientists have developed more powerful tools to aid in the discovery of potent and subtype-selective agents. These advances include greater counter-screen capacity as well as structural information, both experimental and computational, to help guide inhibitor design.

Interest in this class of enzymes intensified after the approval of sildenafil in 1998, followed closely by vardenafil and tadalafil (Fig. 1).[6] These drugs were moderately selective PDE5 inhibitors

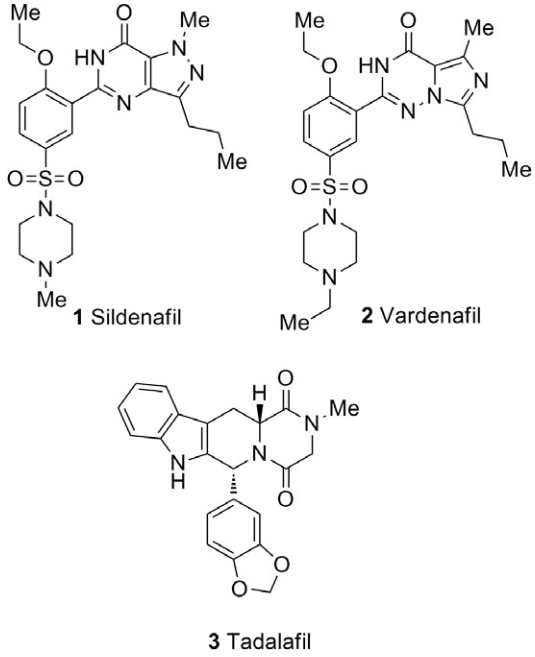

Figure 1. Structures of the first three FDA-approved PDE5 inhibitors.

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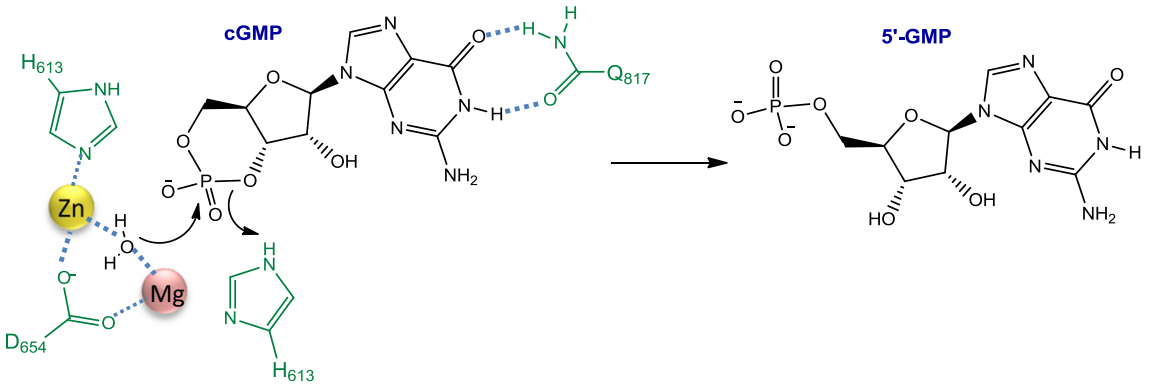

Figure 2. General mechanism for hydrolysis of cGMP by PDE5 showing some of the key residues involved in the binding of the substrate and the metal ions.

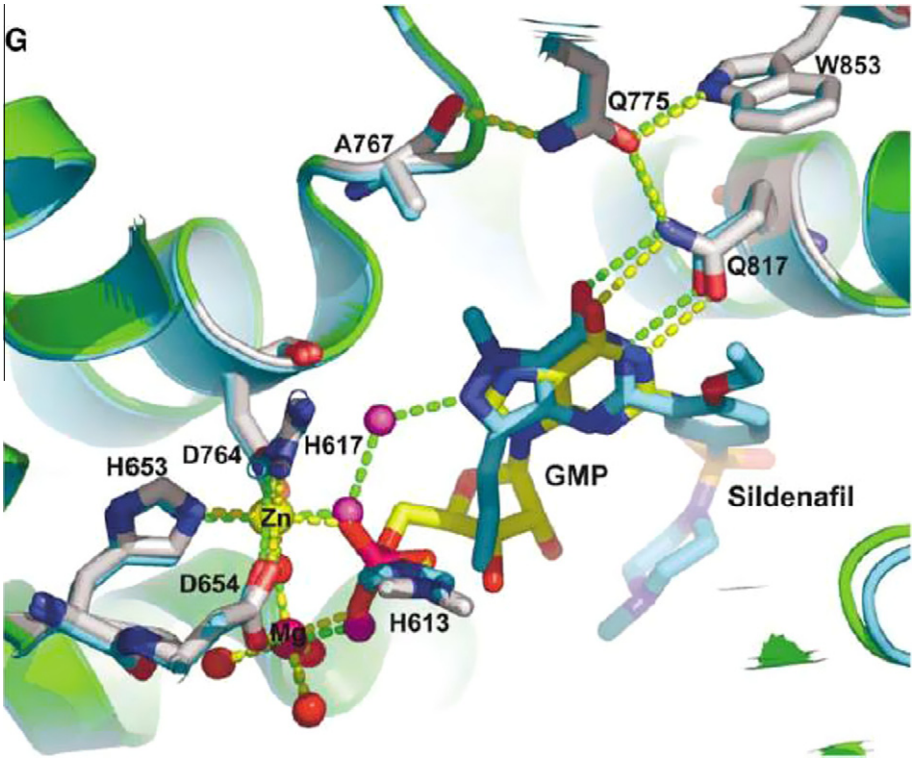

Figure 3. Crystal structures of GMP (yellow) and sildenafil (cyan) bound in the catalytic site of PDE5. (Reprinted from Zhang, K. Y. J. et. al. *Mol. Cell*, **2004**, *15*, 279 with permission from Elsevier.)

initially approved for the treatment of erectile dysfunction. The commercial success of these drugs ushered in a new era in PDE research despite the challenging pre-clinical and clinical history of other PDE targets.

Early research on PDE modulation was non-productive. Selectivity issues were common, often only coming to light after additional sub-types were discovered. Such endeavors often resulted in what seemed like intractable issues, such as emesis with PDE4 inhibitors[7] or increases in hepatic glucose output in PDE3 blocking agents;[8] two popular early targets. With modern technological advances, including library synthesis and structure-based design, achieving selectivity between the PDE families has been shown to be a difficult but reachable goal.[9] Building high levels of selectivity within a family has proven to be much more challenging. Off target activities, whether through inhibition of another PDE isoform, or by blocking the desired PDE in an undesired tissue, will continue to hinder progress unless novel strategies are adopted.

This review will focus on three areas of contemporary phosphodiesterase research:

(1) Beyond PDE5: Recent trends in the discovery and development of selective catalytic domain inhibitors; (2) new insights into PDE structure and function; and (3) novel directions for modulating PDE activity.

The important role of PDEs coupled with the fact that they all contain a well-defined catalytic site, made these enzymes attractive targets for intervention in a wide variety of disease states.[2] Until very recently, nearly all of the work on these targets involved blocking the cAMP or cGMP binding site on the catalytic domain. These catalytic sites contain a base-binding region which positions the cyclic phosphate group toward the zinc and magnesium ions which are surrounded by numerous ordered water molecules involved in the hydrolysis step (Fig. 2). Not surprisingly, many PDE inhibitors resembled the amino purine, or purinone heterocycles found in the endogenous substrates (Fig. 3), but others bear no

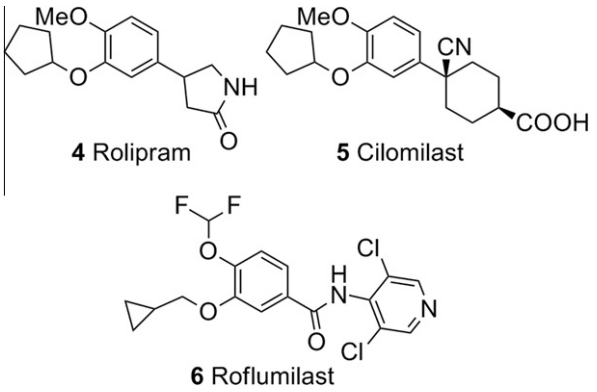

Figure 4. Important PDE4 inhibitors, including roflumilast, the only FDA approved PDE4 inhibitor.

such resemblance. These latter compounds often showed improved selectivity, at least among the different families. The approved PDE5 inhibitors shown in Figure 1 exemplify this point nicely. Both **1** and **2** have purinone-like cores and were poorly selective over PDE6. Compound **3** is structurally unique with high selectivity over PDE6.

Despite the successful launch of several PDE5 and PDE3 inhibitors in the late 1990s, extension of this success to other PDE isoforms has failed to materialize. The majority of ongoing clinical trials of PDE inhibitors are not of NCEs but instead are trials of the already approved PDE5 inhibitors looking at alternative indications ranging from diabetes to cystic fibrosis.[10] In fact, since the year 2000, the only approved non-PDE5 inhibitor has been roflumilast (**6**) in 2011 (Fig. 4). Roflumilast is an inhibitor of PDE4 and is approved for the treatment of respiratory diseases.[11] Its approval came some 35 years after the discovery of the first well known and extensively studied PDE4 inhibitor rolipram (**4**).[12] The development challenges of the PDE4 inhibitors are worth reviewing given that similar issues have plagued the development of inhibitors of other PDE isoforms.

PDE4 is a cAMP-specific PDE with wide tissue distribution and is considered to be one of the main enzymes responsible for controlling intracellular cAMP levels. Like many other targets being pursued in the pharmaceutical industry at the time (i.e., the 1980s), it was believed to be a single enzyme. Based on early research and localization, the two most common diseases targeted for PDE4 inhibitors were depression and inflammation (particularly asthma). The association of PDE4 inhibition and emesis has been well characterized[7] in animals and humans and was one of the main challenges in their development. An enormous effort was expended across the pharmaceutical industry to increase the therapeutic window between efficacy and emetic response. The strategies were largely empirical, but did include some rational approaches such as attempting to limit CNS exposure by property-based design[13] (see cilomilast, Fig. 4) and by minimizing systemic levels of drug by inhalation delivery directly to the lung. Only later was it discovered that PDE4 is made up of four distinct isoforms, A, B, C and D. The current thinking is that the anti-inflammatory efficacy is mediated through PDE4B whereas the emesis is thought to be the result of inhibition of PDE4D in the area postrema of the brain stem via a nor-adrenergic pathway.[7] Despite this knowledge, achieving isoform selectivity within a PDE family has proven to be extremely challenging, even with the aid of co-crystal structures. This fact, coupled with the often wide distribution of PDEs meant that dose-limiting side effects were all too common. To complicate matters further, each PDE subtype has multiple splice variants, the roles of which are not well understood (vide infra). All of these factors complicated the development of PDE inhibitors including roflumilast,[14] and despite its eventual approval, its development was long and expensive only to end up with a somewhat restrictive label.

One approach to overcome these limitations is to focus on a PDE family with limited isoforms, and/or limited tissue distribution. Perhaps by design, or by attrition, the current target receiving the greatest attention is PDE10. PDE10 fulfills both of these requirements being a single isoform family, and showing high expression levels in only a limited number of tissues. It is a dual substrate PDE hydrolyzing both cAMP and cGMP and its localization in the striatum suggested a role in schizophrenia.[15] Well over a dozen companies have filed patents on PDE10 inhibitors over the past five years. Some of the published inhibitors are shown in Figure 5. The Pfizer compound, **7**,[16] is the most advanced, although

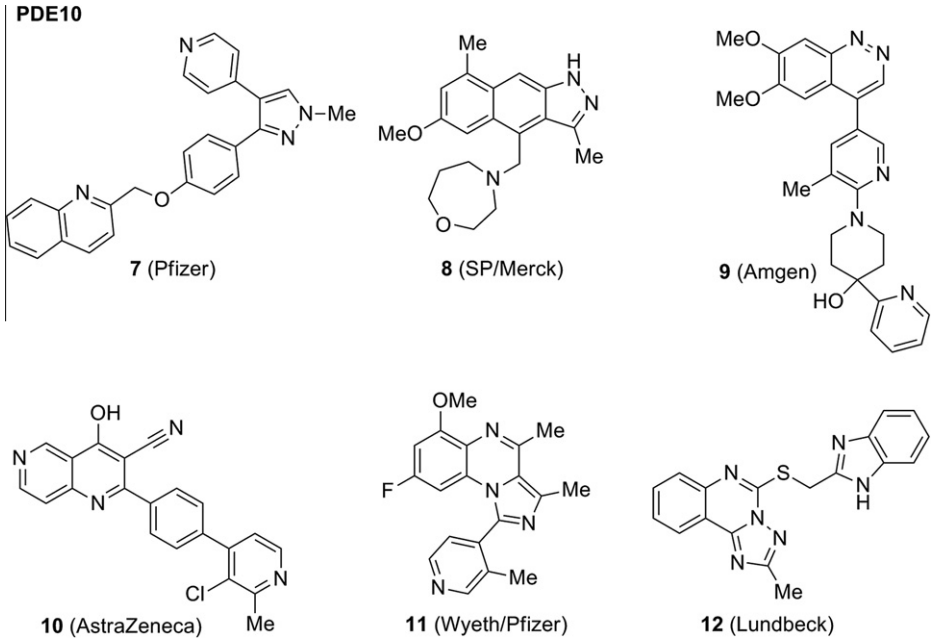

Figure 5. Some of the recently published structures of selective PDE10 inhibitors.

its clinical development appears to have slowed given that after almost six years since entering the clinic, it is still in Phase II.[10] It is currently in a trial looking to block the effects of ketamine as evaluated by both behavioral and imaging methods. Other inhibitors have been reported by Schering-Plough[17] (now Merck, **8**), Amgen[18] (**9**), Astra-Zeneca[19] (**10**), Wyeth[20] (now Pfizer, **11**) and Lundbeck[21] (**12**).

In addition to PDE10, more modest efforts have appeared for several other of the more recently identified PDEs. As a result, selective inhibitors for PDE7,[22–25] PDE8[26,27] and PDE9[28–31] are now available to help further our understanding of these isoforms (Fig. 6). There is no evidence of any appreciable sub-family selectivity within the disclosed PDE7 and PDE8 inhibitors. PDE9, like PDE10, has only one isoform.

Perhaps the most significant advances in PDE research occurred over the past three years and has led to a greater understanding of the structure–function relationship of the enzymes. Specifically, the work unravels the underlying mechanism of how the activity of certain PDEs are controlled. It has long been known that the PDE enzymes exist in two domains: a highly conserved catalytic domain, and a variable regulatory domain.[32] A depiction of the types of regulatory domains of the major PDE families is shown in Figure 7. The role of these regulatory domains has been an area of intense research, and as a result, certain aspects of their function have begun to emerge. For example, the GAF domains, present in five of the families, bind cGMP and/or cAMP modulating the activity of the catalytic domain in an allosteric fashion. Other PDE family members have domains that bind to calmodulin (PDE1) or have

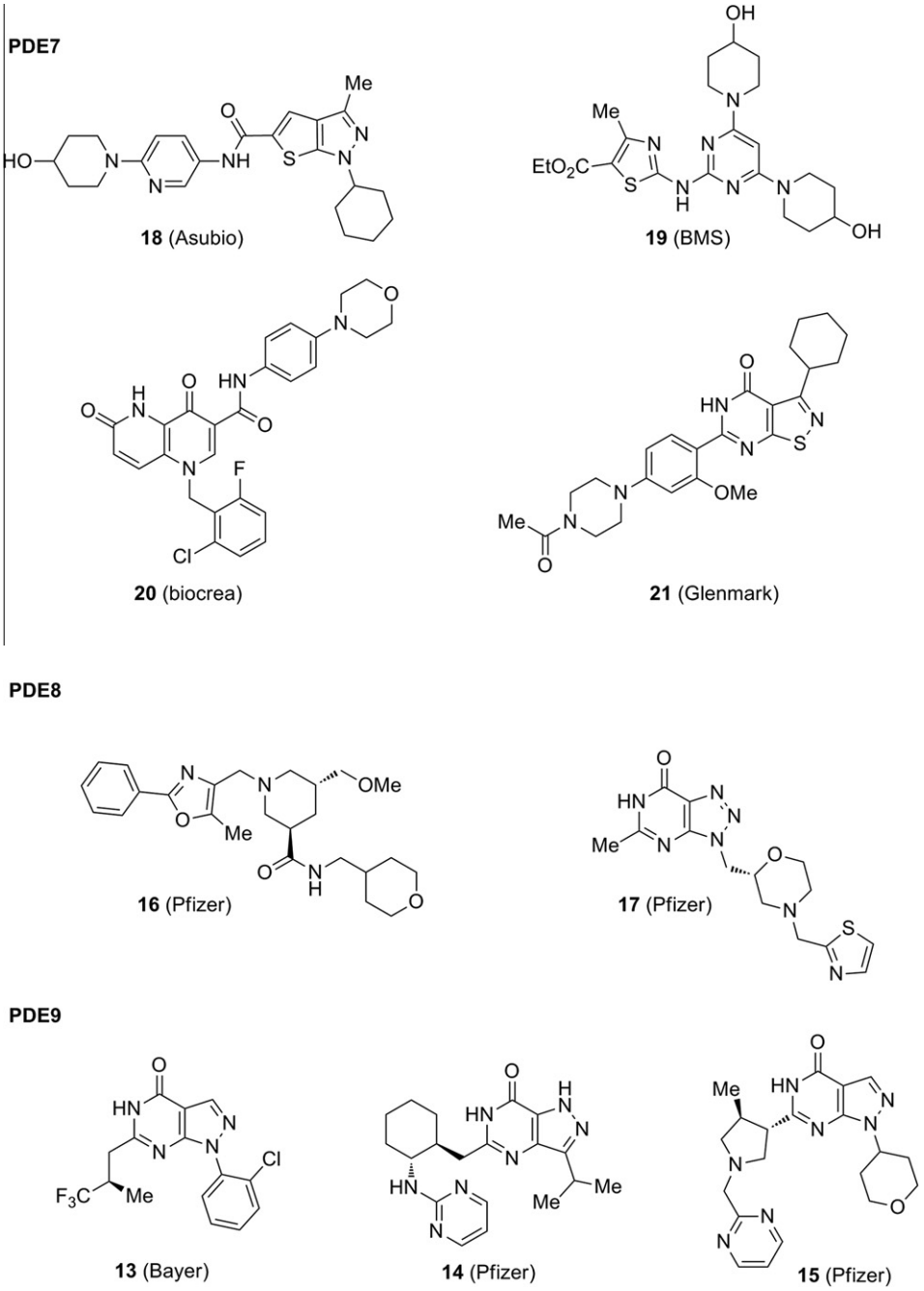

Figure 6. Published structures of inhibitors of some of the more recently discovered PDEs: PDE7, PDE8 and PDE9.

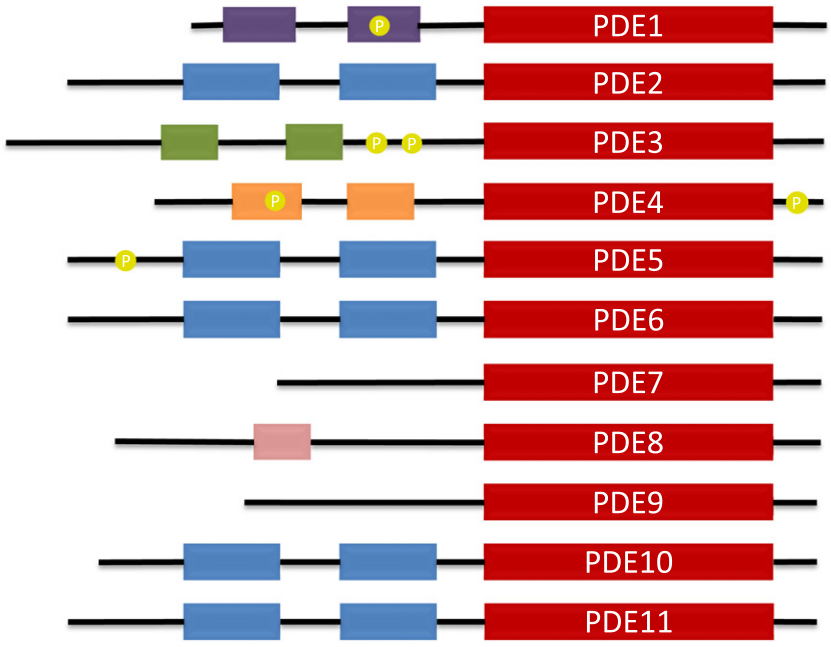

Figure 7. Domains of the eleven PDE families. Catalytic; GAF; Calmodulin binding; Transmembrane (NHR); UCR; PAS; Phosphorylation site.

membrane anchoring regions (PDE3). Despite this intricate interplay between the enzyme sub-units, for research purposes, the regulatory domain was truncated; often for screening, and until recently, universally for structure-based work.

Structure–function studies of the regulatory and catalytic domains progressed largely independently. As mentioned above, they were dissociated prior to crystallization and most of the early work focused on the catalytic domain resulting in structures for most of the 21 isoforms.[33] Structures of the regulatory domains, such as the GAF units are much less common, but have provided important information about cGMP/cAMP binding sites and residues involved in dimerization.[34–36] Indeed, it appeared that both domains formed dimers in the solution and crystal states which supported earlier predictions of dimeric forms of the PDEs based on biochemical observations.[37,38]

The tendency for PDEs to dimerize was intriguing. However, the function of this feature remained elusive until a seminal paper by Pandit et al in 2009.[39] For the first time, a near full length crystal structure of a phosphodiesterase was reported. The structure was of PDE2A which crystallized as a dimer and contained the GAF-A and GAF-B subunits of the regulatory domain. The dimer has a long interface, basically covering the full length of the enzyme. The two monomers of the dimer cross at the juncture between the two domains forming a structure reminiscent of a pair of scissors (Fig. 8).

Another feature of this structure is that a loop that appears to block access to the catalytic site in the apo structures of the catalytic domain but swings away freely in ligand-bound structures (termed the H-loop) is forced into a 'closed' state in the dimer (Fig. 9). This result suggests that dimerization serves to inactivate the PDE, which upon binding of cGMP to the GAF-B domain, results in a conformational change at the dimer interface and the H-loop making the catalytic site accessible. This activation mechanism, which was proposed by the authors, has yet to be confirmed with structural data but is in accordance with biochemical observations (i.e., allosteric regulation with cGMP).

Soon after the PDE2A disclosure, a letter describing the regulation of PDE4D was published also based on crystallographic evidence.[40] In this instance, the regulatory domain has a much more direct role in controlling function by physically blocking access to the catalytic site. The conformational changes involved are in part controlled by phosphorylation of the UCR (Upstream Conserved Region) domain. Such mechanisms reveal alternative strategies for the inactivation or even activation of various PDEs and will be discussed in the last section of this review.

Phosphodiesterases are ancient proteins from an evolutionary perspective. They play a key role in modulating two of the most important signaling molecules in cells. But despite major advances in our understanding of these enzymes and their roles in certain disease states, clinical and commercial success beyond PDE5 has been disappointing. Perhaps this outcome is in part due to the enormous complexity of the PDE systems that has emerged in recent years. Fundamental to this challenge is the interplay between the roles of multiple isoforms and splice variants within a single cell. To reconcile the apparent redundancies among the PDEs one must conclude that the cells possess the ability to carefully control the expression, regulation, dimerization and compartmentalization of various PDEs in a manner that is beyond our complete understanding. It is likely that it is from this new frontier that the next generation of PDE therapeutics will emerge. The days of simply shutting down activity of the catalytic site and hoping for the best appear numbered.

As mentioned earlier, the expression of most PDEs is far too extensive not to expect some off-tissue or off-target side effects that will become dose-limiting in clinical trials. This challenge is particularly relevant when the therapies are targeting cardiovascular, metabolic and CNS diseases where safety is paramount. In order to more selectively and safely modulate cAMP or cGMP levels, a greater understanding of the particular PDE isoform, splice variant, and localization in the disease tissue of interest is required. Careful study of this protein may also provide insights about its regulation as well as any associated proteins which may control its function or compartmentalization. This information, although daunting, could provide many additional opportunities for intervention beyond the catalytic site. Possibilities include interfering with enzyme dimerization, inhibiting phosphorylation, or blocking the activation of the catalytic domain induced by allosteric binding of cAMP or cGMP to the regulatory domain.

Sufficient details have already emerged for selected PDEs to support such strategies. Enzyme splice variants for example, which are alternative combinations of exons, have largely been ignored in

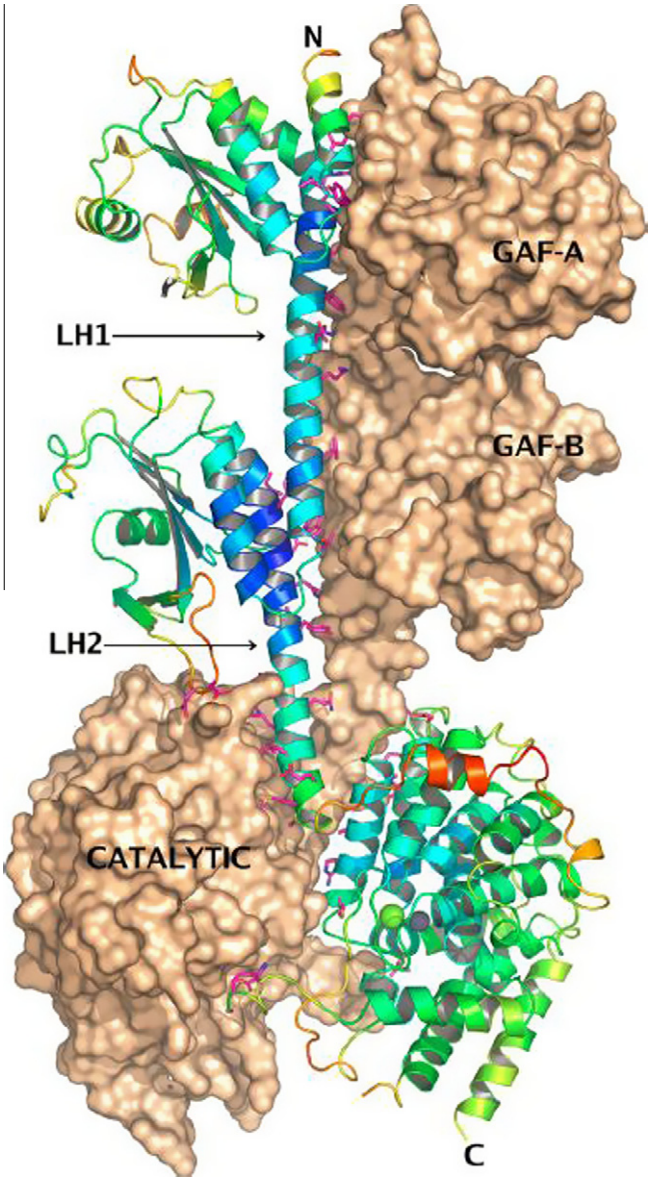

Figure 8. Crystal structure of a nearly full length construct of PDE2A showing a dimeric structure. One monomer is displayed as a surface representation while the second one is in ribbon format. (Reprinted with permission from: Pandit, J.; Forman, M. D.; Fennell, K. F.; Dillman, K. S.; Menniti, F. S. *Proc. Natl. Acad. Sci. U.S.A.*, **2009**, *106*, 18225.)

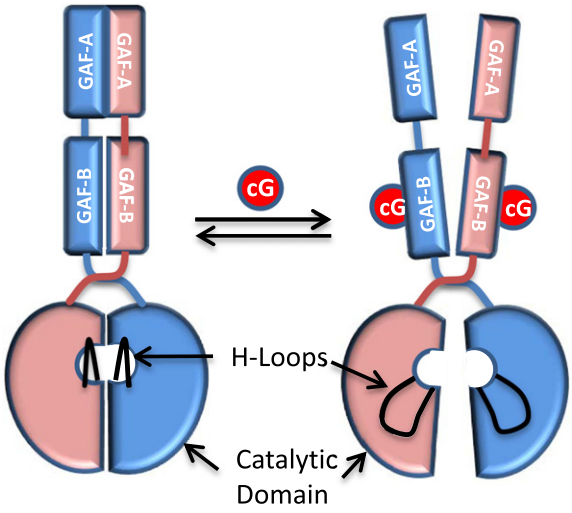

Figure 9. Proposed mechanism for activation of dimeric PDE2A upon cGMP binding to the GAF domain.[39]

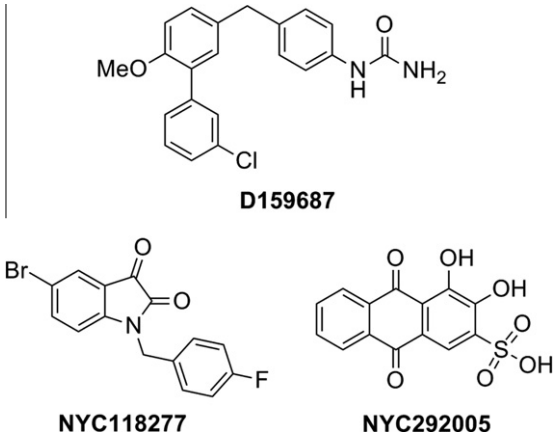

Figure 10. Structures of PDE4D and PDE5 allosteric modulators.

running drug discovery programs. The significance of the splice variants were overlooked despite data suggesting that these post-transcriptional modifications are not just accidents of nature but seem to play a role in the compartmentalization and regulation of specific PDE isoforms.[41] Not unexpectedly, all of these modifications occur in the regulatory domains of the proteins. PDE9, which consists of over 20 splice variants, includes at least one which was shown to be localized to the nucleus.[42,43] Its role there is not yet clear. In addition, variants of PDE4D have been shown to be differentially expressed in various tissues in the rat.[44]

Efforts focusing on non-traditional strategies for PDE modulation have already begun to appear in the literature. A group from deCODE Genetics has optimized a series of allosteric PDE4D partial inhibitors such as D159687 which show cognition enhancing effects and reduced potential for emesis. (Fig. 10). The compounds bind at the interface of the catalytic site and the UCR2 unit of the regulatory domain.[40] Researchers at the University of Tübingen specifically targeted the cGMP allosteric binding sites on the GAF domains of PDE5.[45] For the HTS screen, the GAF-A and GAF-B domains were coupled to adenylate cyclase. Hits such as NYC118277 and NYC292005 were identified, and although not very attractive starting points, do show the validity of the approach. They are now attempting to co-crystalize these compounds with the GAF proteins.

It is appreciated that targeting increased specificity runs contrary to some of the contemporary ideas of drug discovery. Phenotypic screening has gained in popularity in recent years as a way to improve efficacy by targeting multiple pathways with a single agent.[46,47] However, the case for PDEs is somewhat unique given the ubiquitous nature of the signaling molecules they regulate. As such, a more targeted approach toward regulating their activity may prove more successful in the end.

In summary, phosphodiesterases have been shown to be fascinating enzymes. Current approaches for modulating their activity through inhibition of the catalytic site have not been productive despite evidence for their role in certain disease states. New information regarding the structure and function of the individual PDE isoforms, in combination with novel insights about their expression, localization and regulation provide abundant opportunities for the next generation of PDE modulating drugs.

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